Cryptogamie, Algologie 1987, 8 (3) : 201-209

PIT PLUGS AND OTHER ULTRASTRUCTURAL FEATURES OF SYSTEMATIC VALUE IN *RHODOCHAETE PARVULA* (RHODOPHYTA, RHODOCHAETALES)

Curt M. PUESCHEL* and F. MAGNE**

ABSTRACT – *Rhodochaete parvula* Thuret was considered to be unique among the red algae in having pit connections that are not occluded by pit plugs Pit plugs, consisting only of cores, are now demonstrated to occur in this species. Plug caps and cap membranes were not found. Although more specialized than earlier thought, the condition found in *Rhodochaete* is likely to represent the ancestral character state of red algal pit plugs. Unlike most red algae, Golgi bodies in *Rhodochaete* are not closely and consistently associated with mitochondria. One or more peripheral, encircling thylakoids are present in the plastids, another condition likely to represent the ancestral condition of red algae.

RÉSUMÉ. – Le *Rhodochaete parvula* Thuret était jusqu'à ptésent considéré comme possédant des ponctuations intercellulaires dépourvues de synapse. On démontre ici qu'en réalité cette dernière existe bien, mais qu'elle est constituée seulement par le bouchon synaptique, à l'exclusion des capes et des membranes. Bien que plus spécialisée qu'on le pensait, cette condition correspondrait vraisemblablement à l'état ancestral de l'appareil synaptique des algues rouges. Chez *Rhodochaete*, au contraire de la plus grande partie des algues rouges, les corps de Golgi ne sont pas étroitement et constamment associés aux mitochondries. La présence dans les plastes d'un ou plusieurs thylakoïdes enveloppants peut être considérée comme un autre caractère archaïque des algues rouges.

KEY WORDS : Bangiophycidae, chloroplasts, Golgi bodies, pit connections, pit plugs, *Rhodochaete*, Rhodophyta.

INTRODUCTION

Rhodochaete parvula Thuret, the sole member of the Rhodochaetales (Bangiophycidae), is a minute, filamentous species that has been collected only a few times from the Mediterranean Sea and once from the Caribbean Sea (TAYLOR, 1971). The distinctive life history and other aspects of the biology of this alga have been elucidated by MAGNE (1960) and BOILLOT (1969; 1975; 1978).

* Department of Biological Sciences, State University of New York at Binghamton, Binghamton, New York 13901 and ** Laboratoire de Biologie Végétale Marine, Université Pierre et Marie Curie (Paris VI), 7 Quai Saint-Bernard, 75230 Paris Cedex 05.

A unique constellation of characters makes this alga of considerable phylogenetic interest. Its simple reproductive system is appropriate for a bangiophyte, yet *Rhodochaete* also possesses several important florideophycean features. It has apical growth, isomorphic alternation of generations, and pit connections in both gametophyte and sporophyte life history phases.

Although pit connections in *Rhodochaete* are not detectable in fresh specimens, they are sometimes apparent in cytological preparations that cause swelling of cells (MAGNE, 1960). BOILLOT (1978) used electron microscopy to examine pit connections in both life history phases. She reported that the connections were filled by dense material but did not contain well-defined pit plugs. Pit connections between vegetative cells in other red algae invariably contain pit plugs (PUESCHEL & COLE, 1982), unless secondarily removed (e. g. AGHAJANIAN & HOMMERSAND, 1978). In light of the unique pit connections reported by BOILLOT (1978) and the importance of *Rhodochaete* to unravelling the phylogeny of red algae, further ultrastructural study of this species was undertaken.

MATERIALS AND METHODS

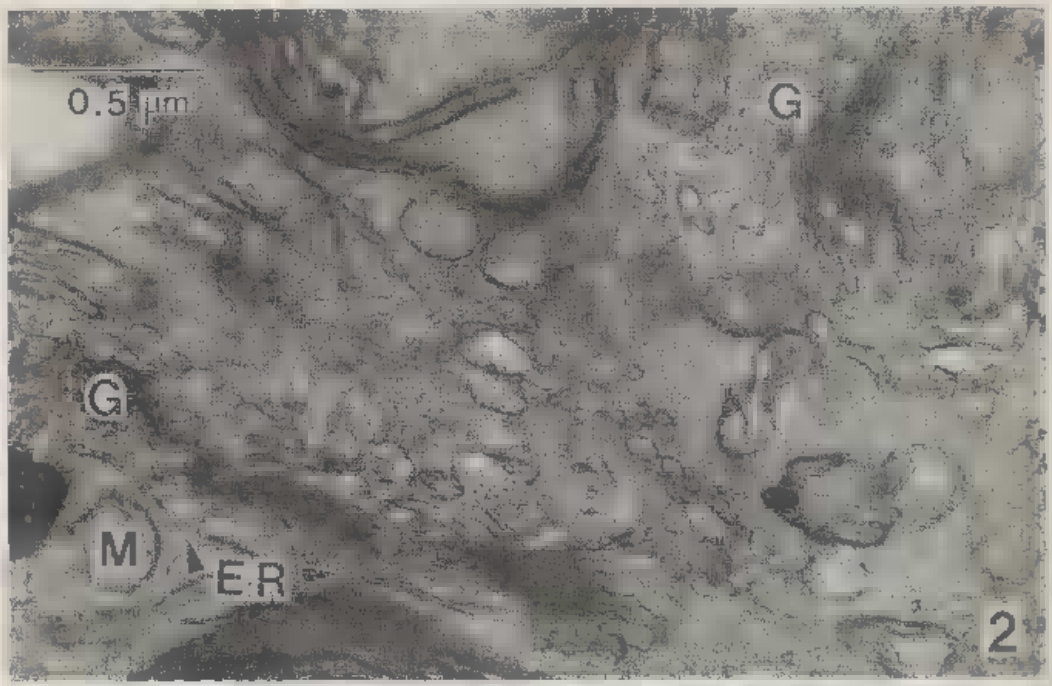
Sporophytes and gametophytes of *Rhodochaete parvula* of a strain isolated from the Mediterranean Sea (Villefranche-sur-mer, France) were cultured in modified Provasoli enriched seawater medium at 14°C on a long day (16-8) photoperiod under cool white fluorescent light at an intensity of 20 $\mu Em^{-2}s^{-1}$. Both sporophytes and gametophytes were fixed in 2.5 % glutaraldehyde, 0.1 M cacodylate, 0.2 M sucrose followed by dilutoin and rinsing in buffer and postfixation in 2 % OsO_4. Other sporophytes were fixed as above except that the postfixative also contained 3 % $K_3Fe(CN)_6$, or the primary fixative solution also contained 2 % tannic acid. In a fourth procedure, 1 % $KMnO_4$ 1 % NaCl was the only fixative used. Before dehydration the filaments were washed in distilled water and pressed onto gelatin-coated glass slides or onto the surface of agar according to CHATTON (1927) to obtain the alignment of filaments in one plane. Dehydration in a graded ethanol series was followed by propylene oxide rinses and infiltration with Epon or Spurr low viscosity resin. Sections were stained with 2 % aqueous uranyl acetate and Reynold's lead citrate.

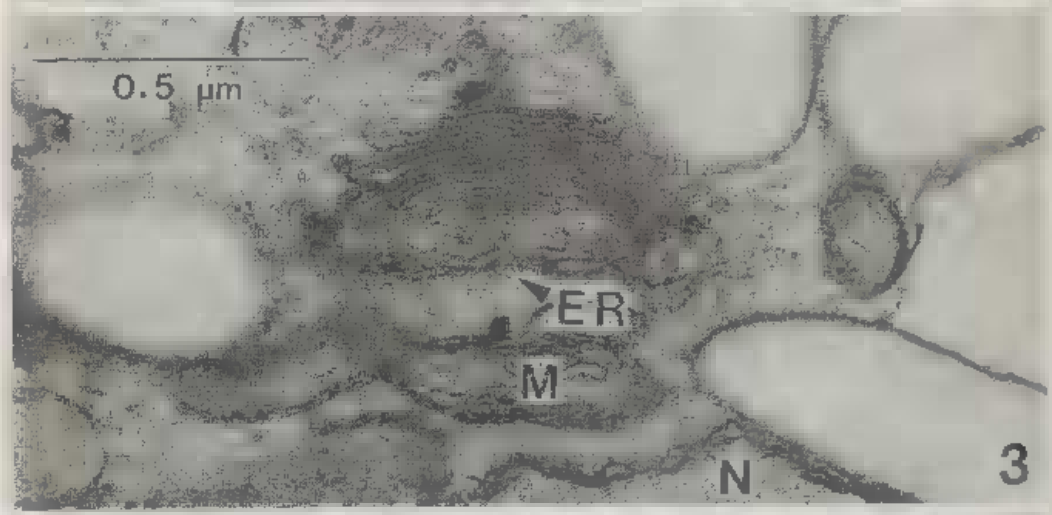
OBSERVATIONS

It is commonly observed that algae freshly collected from the wild yield better ultrastructural preservation than those kept in culture for long periods (PICKETT-HEAPS, 1975). Unfortunately, in this study we did not have fresh specimens with which to make such comparisons. Our approach was to use a variety of fixation procedures, but some problems, especially the shrinkage of cells were common to all fixations. For this reason, measurements given for such dimensions as length of the plugs could be artificially large due to stretching of

Fig. 1 – Apical cell of *Rhodochaete* sporophyte. Golgi bodies (arrows) are particularly numerous near the nucleus (N). Chloroplasts (C) have peripheral encircling thylakoids. Starch grains (S) are numerous in the cytoplasm. Osmium/ferricyanide postfixation.

the connection between the shrinking cells, whereas the values for plug diameters may be somewhat reduced.





The vegetative cells of both sporophytes and gametophytes are elongate, uninucleated cells with numerous chloroplast profiles (fig. 1). The chloroplasts are usually described as numerous and discoid, but serial sectioning was not performed to determine whether these discoid units were connected. Pyrenoids were lacking. Unlike some bangiophytes, the chloroplasts in *Rhodochaete* had one or more peripheral thylakoids that were parallel to the chloroplast envelope and thus encircled the other thylakoids (figs. 1 and 2). This was true of chloroplasts of both sporophytes and gametophytes.

The cell wall was densely fibrillar and evenly thick except at the apical end of the filament (fig. 1). The wall covering the dome-shaped tip of the apical cell was thinner and considerably less dense, as might be expected for a thallus with apical growth. Starch grains were numerous and variable in outline. Microbody-like structures were observed in proximity to the nucleus in some cells.

Golgi bodies were numerous, especially in apical cells (fig. 2). Although dictyosomes were often in proximity to the nucleus, the relationship was not an intimate one (fig. 3). Endoplasmic reticulum (ER), not the nuclear envelope, occupied the forming (cis) face of the dictyosome. This Golgi-ER relationship was a consistent one and the two organelles were usually separated by a region rich in vesicles, presumably, in transit between the two (figs. 2-4). Mitochondria were often nearby, but they did not occupy the cis face of the cisternal stack (fig. 3), as typically is found in the florideophytes. In addition to their perinuclear position, Golgi bodies were also common near the cell surface, with the cis face of the Golgi separated from the plasmalemma only by a cisterna of parietal ER (fig. 4).

The sporophyte and gametophyte generations of *Rhodochaete* differed conspicuously in the dimensions of their pit plugs and subtly in the substructure of the plugs (figs. 5 and 6). The length of the plugs in sporophytes ranged from about 1.2 to 1.8 μm, which was about twice the size of plugs in gametophytes. The widest portion of the plugs was near the ends where they contacted the cytoplasm (figs. 5 and 6). The ends had a diameter of about 0.35 μm in sporophytes and 0.2 μm in gametophytes. From the ends, the plug tapered towards the middle. The narrowest portion of the plug often was cylindrical (figs. 5 and 6) rather than just a constriction. In sporophytes, this constricted region was up to 0.3 μm long and it had a relatively constant diameter in the range of 0.1 to 0.15 μm. In gametophytes, the constriction was not as long and the upper range of its diameter was 0.1 μm. The midpoint of the cylindrical portion of the plug

---

Fig. 2 – Golgi bodies (G) have cisternae of ER (ER) at their forming faces. Mitochondria (M) may be nearby, but they are not intimately associated with the Golgi. Osmium/ferricyanide postfixation. Fig. 3 – A Golgi body is present near the nucleus (N), but ER is at the forming face of the dictyosome. Osmium/ferricyanide postfixation. Fig. 4 – In a negative image created by tannic acid, ER near the cell surface occupies the forming face of the dictyosome. Mature Golgi vesicles are cut off towards the center of the cell.

Fig. 5 – Pit plug of a sporophyte thallus, postfixed with osmium/ferricyanide. Note the dense, cylindrical mid-region. The plasmalemma (arrows) is continuous from cell to cell along the sides of the plug. Fig. 6 - Pit plug of gametophyte, osmium postfixation. The dense central portion of the plug is penetrated by a channel of less electron-density. No indication of the presence of plug caps or cap membranes is found in either phase of the life history.

sometimes showed a slight bulge (fig. 5). The constricted portion of the plug was generally more electron dense than the wider regions closer to the cytoplasm (figs. 5 and 6). In the gametophytes, a narrow shaft of low density appeared to penetrate into the denser matrix of the constriction (fig. 6). This was less apparent in sporophytes (fig. 5).

The plasmalemma, as in all pit connections, lined the sides of the plug core and was continuous from cell to cell (fig. 5). However, no membrane was found between the cytoplasm and the plug core, nor were plug caps present in any of the fixation regimes used, either in gametophytes or sporophytes. Some plugs detached completely from the adjacent cells they once connected and seemed to suffer little distorsion or stretching. In other preparations, the persistence of the attachment and the shrinkage of the cells appeared to stretch the plugs. In some such cases, fine filaments, which may represent a component of the cytoskeleton, were observed to stretch from the end of the plug into the cytoplasm.

DISCUSSION

The present study demonstrates that pit plugs are present in *Rhodochaete parvula*, contrary to the report of BOILLOT (1978) that the pit connection is occupied by compacted protoplasm. This finding is of considerable importance for it indicates that the structures in *Rhodochaete* are homologous to the pit plugs in other red algae and presumably are similar in their chemical composition and mode of deposition.

In florideophytes, the pit plugs may have a complex organization including a plug core, plug caps of one or two layers, and associated membranes (PUESCHEL & COLE, 1982). In contrast, the pit plugs in *Rhodochaete* are structurally simple; they consist of a plug core, without caps and without a membrane between the core and the cytoplasm. The *Rhodochaete* plugs, which are the minimum level of organization identifiable as a pit plug, are consistent with expectations generated by the relatively simple plugs in the Bangiales, which have only a slender cap layer and no cap membrane (PUESCHEL, 1987). They are also strikingly similar in both size and basic organization to the pit plugs recently demonstrated ultrastructurally in *Compsopogon*, a freshwater bangiophyte (SCOTT, THOMAS & SAUNDERS, in manuscript). The commonality of plug structure in these two primitive taxa lends support to the notion that a plug core without caps or cap membranes is the ancestral condition of the pit plug.

Cells of the sporophyte generation of *Rhodochaete* are larger than those of the gametophytes. It is not too surprising then that the pit plugs in gametophytes are smaller. A relationship between cell size and plug dimensions is apparent in many red algae. A more unusual feature of *Rhodochaete* is the dense matrix in the cylindrical midsection of the plug. In gametophytes, the dense material is not solid; a shaft of less dense material penetrates the denser matrix. More study of both generations is necessary to evaluate this particular feature.

The close spatial association of the forming faces of the Golgi bodies with mitochondria is a distinctive feature of red algal cell structure. *Rhodochaete* does not follow this general pattern. The Golgi are consistently paired with ER; any relationship with mitochondria is less consistent and intimate. SCOTT (1984) did a survey of Golgi associations that showed a correlation between the types of organelle associations and taxonomic groupings. Whereas the florideophytes all have an affiliation of Golgi and mitochondria, several families among the bangiophytes lack this feature. The present study demonstrates that the Rhodochaetales fall among those groups whose Golgi bodies are associated with neither mitochondria nor nuclei.

Elucidating the pattern of thylakoid disposition in *Rhodochaete* adds to our perspective on the organization of red algal chloroplasts. At one time, based on a relatively small taxonomic sample, the absence of a peripheral encircling thylakoid was believed to be associated with the «lower Rhodophyceae», whereas the more advanced red algae were believed to have one or more peripheral thylakoids (BISALPUTRA, 1974). Many additional taxa have been examined

since that view was expressed. The Rhodochaetaceae is the last family of the bangiophytes to be characterized. It is now clear that the absence of peripheral thylakoids is a specialization found in the gametophytes of the Bangiaceae (COLE & CONWAY, 1975) and some members of the Porphyridiales (GANTT & CONTI, 1965; EVANS, 1970; SCOTT, 1986). Other members of the Porphyridiales do have encircling thylakoids (eg. DEASON, BUTLER, & RHYNE, 1983; FORD, 1984) and the conchocelis phase of the bangialean life history shares this feature (COLE & CONWAY, 1975). The Boldiaceae (SHEATH & HYMES, 1980), Erythropeltidaceae (MCBRIDE & COLE, 1971), Compsopogonaceae (NICHOLS, RIDGWAY, & BOLD, 1971), and Rhodochaetaceae all have peripheral thylakoids. This configuration may prove to be the primitive condition of thylakoid disposition in red algae.

The wall of the apical cell of *Rhodochaete* is conspicuously thinner at the tip, presumably reflecting tip growth. This condition is similar to that found in the conchocelis developing from germinating carpospores of *Porphyra variegata* (PUESCHEL & COLE, 1985).

If one accepts the entirely plausible proposition that unicellularity, absence of sex, and absence of pit plugs are all derived conditions in extant bangiophytes, then it is clear that *Rhodochaete* is perhaps the most phylogenetically important red alga known, a view previously expressed by CHADEFAUD (1963).

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the technical assistance of H. Eichelberger and the helpful suggestions of J. Scott. This study was supported by NSF grant BSR 83-20598 to C. Pueschel.

REFERENCES

AGHAJANIAN J.G. and HOMMERSAND M.H., 1978 – The fine structure of the pit connections of *Batrachospermum sirodotii* Skuja. *Protoplasma* 96 : 247-265.

BISALPUTRA T., 1974 – Plastids. *In* : STEWART W.D.P. (Ed.), *Algal Physiology and Biochemistry*. Berkeley, Univ. of Calif. Press, pp. 124-160.

BOILLOT A., 1969 – Sur le cycle de *Rhodochaete parvula* Thuret. *Compt. Rend. Hebd. Séances Acad. Sci.* 269 D : 2205-2207.

BOILLOT A., 1975 – Cycle biologique de *Rhodochaete parvula* (Thuret) (Rhodophycées, Bangiophycidées). *Pubbl. Staz. Zool. Napoli* 39 : Suppl., 67-83.

BOILLOT A., 1978 – Les ponctuations intercellulaires du *Rhodochaete parvula* Thuret (Rhodophycées, Bangiophycidées). *Rev. Algol.* n. s. 13 : 251-258.

CHADEFAUD M., 1963 – Sur les organes reproducteurs et l'évolution des Rhodophycées : une hypothèse. *Rev. Algol.* n. s. 6 : 255-269.

CHATTON E., 1927 – La microinclusion à l'agar-paraffine, technique protistologique et embryologique. *Bull. Histol. Appl.* 4 : 355-363.

COLE K. and CONWAY E., 1975 – Phenetic implications of structural features of the perennating phase in the life history of *Porphyra* and *Bangia* (Bangiophyceae, Rhodophyta). *Phycologia* 14 : 239-245.

DEASON T.R., BUTLER G.L., and RHYNE C., 1983 – *Rhodella reticulata* sp. nov., a new coccoid rhodophytan alga (Porphyridiales). *J. Phycol.* 19 : 104-111.

EVANS L.V., 1970 – Electron microscopical observations on a new red algal unicell, *Rhodella maculata* gen. nov., sp. nov. *Brit. Phycol. J.* 5 : 1-13.

FORD T.W., 1984 – A comparative ultrastructural study of *Cyanidium caldarium* and the unicellular red alga *Rhodosorus marinus*. *Ann. Bot.* 53 : 285-294.

GANTT E. and CONTI S.F., 1965 – The ultrastructure of *Porphyridium cruentum*. *J. Cell Biol.* 26 : 365-381.

MAGNE F., 1960 – Le *Rhodochaete parvula* Thuret (Bangioidée) et sa reproduction sexuée. *Cah. Biol. Mar.* 1 : 407-420.

MCBRIDE D.L. and COLE K.M., 1971 – Fine structural studies on several Pacific Coast representatives of the Erythropeltidaceae (Rhodophyceae). *Proc. Int. Seaweed Symp.* 7 : 159-164.

NICHOLS H.W., RIDGWAY J.E., and BOLD H.C., 1966 – A preliminary ultrastructural study of the freshwater red alga *Compsopogon*. *Ann. Missouri Bot. Gard.* 53 : 17-27.

PICKETT-HEAPS J.D., 1975 – *Green Algae. Structure, Reproduction and Evolution in selected genera.* Sunderland, Massachusetts, Sinauer Associates, 606 p.

PUESCHEL C.M., 1987 – Absence of cap membranes as a characteristic of some red algal orders. *J. Phycol.* 23 : 150-156.

PUESCHEL C.M. and COLE K.M., 1982 – Rhodophycean pit plugs : an ultrastructural survey with taxonomic implications. *Amer. J. Bot.* 69 : 703-720.

PUESCHEL C.M. and COLE K.M., 1985 – Ultrastructure of germinating carpospores of *Porphyra variegata* (Kjellm.) Hus (Bangiales, Rhodophyta). *J. Phycol.* 21 : 146-154.

SCOTT J., 1984 – Electron microscopic contributions to red algal phylogeny. *J. Phycol.* 20 : Suppl., 6.

SCOTT J., 1986 – Ultrastructure of cell division in the unicellular red alga *Flintiella sanguinaria*. *Canad. J. Bot.* 64 : 516-524.

SHEATH R.G. and HYMES B.J., 1980 – A preliminary investigation of the freshwater red algae in streams of southern Ontario, Canada. *Canad. J. Bot.* 58 : 1295-1318.

TAYLOR W.R., 1971 – Notes on algae from the tropical Atlantic Ocean - V. *Brit. Phycol. J.* 6 : 145-156.